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Hemisynthesis and absolute configuration of novel 6-pentyl-2*H*-pyran-2-one derivatives from *Trichoderma* spp.

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ABSTRACT

A comparative study of the secondary metabolism of two *Trichoderma* spp. with that of the *Thctf1* transcription factor gene null mutant of *Trichoderma harzianum* 34 was carried out in order to deepen our knowledge of the biosynthetic pathway and mode of action of 6-pentyl-2*H*-pyran-2-one (**1**) and its derivatives as biocontrol agents. New isolated metabolites have shed light on the detoxification mechanism of 6-pentyl-pyranone by *Trichoderma* spp. All new compounds were synthesized and their stereoisomer characterized. The absolute configuration of 6-[(1′*R*,2′*S*)-dihydroxypentyl]-2*H*-pyran-2-one and 6-((1′*S*,2′*R*)-2′-propyloxiran-1-yl)-2*H*-pyran-2-one was determined by NMR analysis of the corresponding Mosher's esters.

1. Introduction

Biological control provides an alternative to the use of synthetic pesticides with the advantages of greater public acceptance and reduced environmental impact. Many of the soils that naturally suppress plant diseases are rich in organic matter that supports the growth of beneficial microorganisms. Use of these microorganisms as biological control agents seeks to restore the beneficial balance of natural ecosystems, which is often lost in crop situations.[1]

Trichoderma species are free-living fungi, which are highly interactive in root, soil, and foliar environments. Considered to be eager colonizers and particularly invasive fungi, their mechanisms of biocontrol include mycoparasitism, competition for nutrients and antibiosis, as well as plant growth promotion[2] and plant defense signaling activation.[3]

This dominance is achieved by biosynthesizing a wide array of secondary metabolites, transforming a great variety of natural and xenobiotic compounds[4] and producing varied fungal cell wall degrading enzymes (CWDEs) such as chitinases, proteases, or glucanases.[5]

Trichoderma species and their metabolites have been the focus of considerable attention over the last several years as potential biological control agents of a variety of plant pathogenic fungi.[6,7]

It is clear that the ability of *Trichoderma* to inhibit the growth of other fungi is probably due to the combined action of CWDEs together with the capacity of *Trichoderma* to produce different secondary metabolites.[8] The secondary metabolites with antibiotic activity produced by *Trichoderma* have been classified into different groups based on their biosynthetic origin or their chemical structure.[9,10]

Some metabolites have been associated with the antagonistic ability of *Trichoderma*, but none of them has been identified as the sole agent responsible for it. One of the first volatile antifungal compounds isolated from *Trichoderma* species was 6-pentyl-2*H*-pyran-2-one (6PP) (**1**) from *Trichoderma viride,*[11] which has subsequently been isolated from several *Trichoderma* species.[12,13]

6PP is responsible for the 'coconut aroma' associated with certain strains of *Trichoderma*,[14] and it is also well known that it can inhibit the growth of a range of phytopathogens, such as *Botrytis cinerea*, *Rhizoctonia solani*, *Armillaria mellea*,[15] *Penicillium expansium*, and *Fusarium graminearum*,[16] and also affects the biomass growth of *Trichoderma* species.[17] Several of these fungi are able to metabolize **1** into products of reduced toxicity to themselves, as a part of the fungal detoxification mechanism.[16,18,19]

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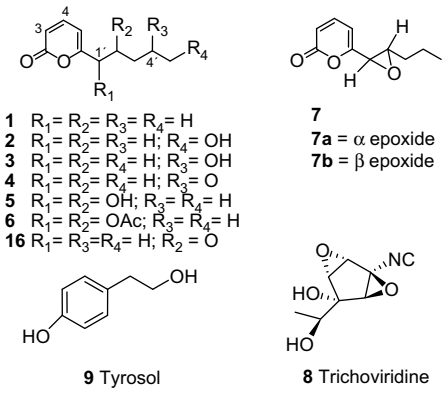
Following up on our project oriented toward the study of the secondary metabolism of *Trichoderma* spp. and the growth inhibition mechanism exerted by these species on phytopathogen fungi, Rubio and co-workers[20] have isolated, characterized, and functionally analyzed, by gene disruption, the first *Thctf1* transcription factor gene related to 6-pentyl-2*H*-pyran-2-one production in *Trichoderma harzianum*. In addition, a comparative study on the secondary metabolism of two wild *Trichoderma* spp. with that of the *Thctf1* gene null mutant strain was carried out. This paper deals with the characterization, synthesis, and absolute configuration of the new metabolites isolated from both wild strains. Some considerations regarding the detoxification mechanism of 6PP by the fungus *B. cinerea* are likewise reported.

2. Results and discussion

In order to enhance our knowledge in the biosynthetic pathway and in the mode of action of 6-pentyl-2*H*-pyran-2-one (**1**) and its derivatives as biocontrol agents, we have conducted a comparative study on the secondary metabolism and the production of pentylpyrone derivatives by the null transformant and two wild-type strains of *Trichoderma*: *T. harzianum* CECT 2413, the parental strain of the *Thctf1* null mutant, and the wild strain *T. viride* UCA 06.

Study of the extract obtained from the broth of *Thctf1* null mutant (see Experimental section) did not produce 6PP or any of its derivatives and exerted a lower antimicrobial effect than the wild-type strain. These results linked the transcription factor *Thctf1* gene of *T. harzianum* to the production of secondary metabolites and antifungal activity against plant pathogenic fungi.

Fermentation and isolation procedures of the products obtained from the broth of both wild-type of *Trichoderma* strains were carried out as described in Experimental section. Under these conditions, in addition to **1**, compounds **3**, **5** and **2**–**4**, **7**, retaining the pyrone ring (Scheme 1), were isolated from *T. harzianum* T34 and *T. viride* UCA 06, respectively.



Scheme 1. Metabolites isolated from the *Trichoderma* spp.

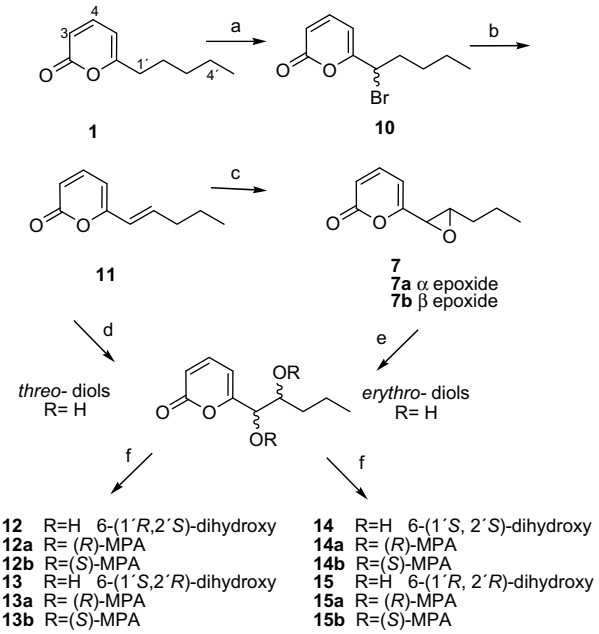
Spectroscopic data for structures **2**, **3**, and **4** coincided with those described in the literature for compounds 6-(5-hydroxypentyl)-2*H*-pyran-2-one (**2**),[16,18] 6-(4-hydroxypentyl)-2*H*-pyran-2-one (**3**),[18] and 6-(4-oxopentyl)-2*H*-pyran-2-one (viridepyronone) (**4**).[21] Products **2** and **3** have previously been described as products resulting from the biotransformation of 6-pentyl-2*H*-pyran-2-one (**1**) by several fungal isolates,[16,18] but not as secondary metabolites from *Trichoderma* species. Compound **5** was obtained as a colorless oil with the molecular formula $C_{10}H_{14}O_4$, obtained from its HRMS and corroborated by ^{13}C NMR data. The ^1H NMR and ^{13}C NMR spectra showed that it retained the pyrone ring and presented signals characteristic of two protons geminal to hydroxyl groups at δ 4.24 (br s, 1H, H-1′) and 3.98 (m, 1H, H-2′).

The proposed structure **5** was confirmed by its diacetyl derivative (**6**), which, in addition to the signals corresponding to the methyl of acetate groups, 2.03 (s) and 2.14 (s), showed two characteristic signals of protons geminal to the acetoxy groups deshielded at δ 5.56 (d, 1H, H-1′) and 5.28 (ddd, 1H, H-2′), respectively. Furthermore, structure **5** was supported by homo- and heteronuclear-2D-correlation experiments. The stereochemistry at both hydroxyl groups was confirmed by chemical correlation as indicated below.

From the fermentations of *T. viride* UCA 06 carried out with the secondary metabolites elicitor (see Experimental section), in addition to **2**–**4**, compounds **1** and **7**–**9** were also isolated. Spectroscopic data for product **8** coincided with those previously reported for a characteristic metabolite of this species, trichoviridine[22] (**8**). Tyrosol (**9**)[23] is a known compound, which is reported here for the first time as a secondary metabolite isolated from *T. viride*.

Epoxy-compound **7** was isolated as a colorless oil, with an M^+ peak at *m*/*z* 180 and gave rise to a ^{13}C NMR spectrum consistent with the molecular formula $C_{10}H_{12}O_3$. The ^1H NMR spectrum was close to that of 6-pentyl-2*H*-pyran-2-one (**1**), but the appearance of two signals at 3.17 and 3.36 ppm in the ^1H NMR spectrum and at 61.1 and 54.3 ppm in the ^{13}C NMR spectrum (H-2′ and H-1′, respectively) showed that **7** was a 6-pentyl-2*H*-pyran-2-one epoxy derivative. Compound 6-(2′-propyloxiran-1-yl)-2*H*-pyran-2-one (**7**), is reported here for the first time.

In order to assign the absolute configuration of new compounds **5** and **7**, we proceeded to the synthesis and characterization of the stereoisomers of both compounds, as indicated in Scheme 2.



Scheme 2. (a) NBS, $(PhCO)_2O_2$; (b) LiBr/Li_2CO_3; (c) *m*-CPBA; (d) OsO_4/NMO; (e) $HClO_4$ (2 N); (f) DMAP, DCC and (*R*)-MPA or (*S*)-MPA.

The synthesis started with commercially available 6PP (**1**), which was quantitatively converted into 6-(1′-bromopentyl)-2*H*-pyran-2-one (**10**) upon treatment with *N*-bromosuccinimide (NBS) and a catalytic amount of benzoyl peroxide in CCl_4.[24]

Compound **10** was obtained as a colorless oil with the molecular formula $C_{10}H_{13}BrO_2$, determined from its HRMS and corroborated by ^{13}C NMR data. The ^1H NMR and ^{13}C NMR spectra showed that it retained the pyrone ring and presented a characteristic signal at δ 4.50 (t, 1H, *J*=7.7 Hz, H-1′) and 48.0 (d, C-1′), respectively, according to structure **10**.

The formation of side-chain olefin was carried out in basic medium ($LiBr/LiCO_3$)[25] affording **11**[26] at a yield of 75%. The former represented the starting material for the synthesis pathway leading to the *threo*- and *erythro*-diol derivatives **12**–**15**, and both epoxy enantiomers **7a** and **7b**.

The racemic mixture of *threo*-1′,2′-diol **12**/**13** was obtained at a yield of 80% by dihydroxylation using common osmium tetroxide/trimethylamine *N*-oxide dehydrate.

Moreover, the racemic mixture of *erythro*-1′,2′diols **14**/**15** was afforded by two subsequent reactions, epoxidation of the olefin **11** with *m*-chloroperbenzoic acid (m-CPBA) to give epoxide **7** (81%) and ring opening of this by treatment with perchloric acid (6%) in dimethoxyethane (DME). The *erythro*-diol racemic mixture was obtained at a yield of 70%, along with product **16** (20%) and 5% of recovered starting material.

Reaction of the *threo*-diol racemic mixture with the (*R*)-α-methoxy phenyl acetic acid ((*R*)-MPA) and (*S*)-α-methoxy phenyl acetic acid ((*S*)-MPA), separately, and DCC[27] gave the corresponding Mosher diastereoisomer esters, which were easily separated by HPLC to yield esters **12a**, **12b**, **13a**, and **13b**.

Similarly, the corresponding Mosher esters were obtained from the racemic *erythro*-diols with (*R*)-MPA and (*S*)-MPA, respectively, as outlined in Scheme 2 to give esters **14a**, **14b**, **15a**, and **15b**.

The modified Mosher's method has been proven useful for acyclic 1,2-glycols possessing simple alkyl groups[28] and more recently Riguera's group[29] predicted four general patterns for 1,2-diols. In accordance with this method, we undertook the analysis of ^{1}H NMR spectra of all di-MPA esters already prepared to determine the absolute configuration of the two vicinal carbinol carbons C-1′/C-2′. As pointed out in Table 1, the positive value of $\delta\delta^{RS}$ ($\delta\delta^{RS}[H\alpha(1')]=\delta^{R}[H\alpha(1')]-\delta^{S}[H\alpha(1')]$) for H-1′ and H-2′ indicated 1′*R*,2′*S* configuration in **12a** and **12b**, whilst the negative value of $\Delta\delta^{RS}$ showed (1′*S*,2′*R*) configuration in **13a** and **13b**. Similarly, the (1′*S*,2′*S*) configuration in both **14a** and **14b** and the (1′*R*,2′*R*) configuration in **15a** and **15b** were also established by the Mosher ester method.

Hydrolysis of the corresponding di-MPA ester diastereoisomers, separately, under alkaline conditions (K_2CO_3, MeOH),[30] produced single enantiomers **12**–**15**, which were purified by SiO_2 flash chromatography and isolated in their pure forms. Optical rotations of synthetic enantiomers **12**–**15** were found to be those indicated in Experimental section. The absolute configuration of the naturally occurring diol derivative **5** ($[\alpha]_D^{25}$ +122.5 (*c* 0.11, MeOH)) was established as (1′*R*,2′*S*) by comparison with the optical rotations showed by the synthetic enantiomer (**12**), $[\alpha]_D^{25}$ +120.6 (*c* 0.15, MeOH) confirming its structure as 6-[(1′*R*,2′*S*)-1′,2′-dihydroxypentyl]-2*H*-pyran-2-one.

On the other hand, epoxy enantiomers **7a** and **7b** were separated by HPLC (hexane/isopropanol 95:5), using a chiral column, from the racemic mixture obtained from the treatment of **11** with *m*-CPBA (see Experimental section). The optical activity was measured for both enantiomers and compared with that displayed by the natural epoxide obtained from *T. viride*. The enantiomer **7a**, which presented the same α_D as the natural epoxide, was treated with perchloric acid (6%) in dimethoxyethane (DME) and the physical and spectroscopic constants of the former dihydroxy derivative were measured and compared with those exhibited by diols **12**–**15**. The *erytro*-dihydroxy derivative **15** was obtained indicating that the natural epoxide corresponded to 6-((1′*S*,2′*R*)-2′-propyloxiran-1-yl)-2*H*-pyran-2-one (**7a**).

As mentioned above, compounds **2**–**4** and several 6PP hydroxylated derivatives in position C-1′-C-5′ were isolated as a result of microbial transformation of **1** by selected fungal isolates,[16,18,19] including *B. cinerea*. These authors concluded that different phytopathogenic fungi have the ability to transform a potentially toxic secondary metabolite, from a competitive fungus, into products of reduced toxicity to itself, indicating the existence of a detoxification mechanism in a number of fungal genera.[18] With this in mind, we have undertaken the study of a possible self-detoxification mechanism of **1** by *Trichoderma* spp.

Hence, compounds **1**–**7** were evaluated for their in vitro antifungal activity against the phytopathogen fungus *B. cinerea*, following the poisoned food bioassay.[31] The commercial fungicide dichlofluanid was used as a standard for comparison in this test. The results showed (see Experimental section) that the oxidized metabolites **2**–**5** were inactive or displayed very low fungicide activity at 100 ppm and, in general, they displayed lower fungicide activity than compound **1**. These results seem to indicate that *Trichoderma* spp. possesses a mechanism whereby to detoxify compound **1** to the less toxic compounds **2**–**5**, by hydroxylation of positions C′1-C′-5 of compound **1.**

Table 1
$\Delta\delta^{RS}[H\alpha(X')]=\delta^{R}[H\alpha(X')]-\delta^{S}[H\alpha(X')]$ for compounds **12**–**15**

Compounds	$\Delta\delta^{RS}[H\alpha(1')]$	$\Delta\delta^{RS}[H\alpha(2')]$
12	+0.08	+0.05
13	−0.095	−0.061
14	+0.11	−0.214
15	−0.11	0.212

3. Conclusions

In conclusion, preparation of enantiomeric pure compounds **7a** and **7b**, and **12**–**15** allowed us to determine the absolute configuration of the new metabolites isolated from *Trichoderma* spp. The isolated metabolites, together with the results obtained from fungicide bioassays have revealed an interesting detoxification mechanism, shedding light on the secondary metabolism of *Trichoderma* spp. The existence of this microbial detoxification pathway for the fungistatic agent 6PP (**1**) suggests that it might not persist in the environment for a prolonged period and it would be of interest as an ecological fungicide–fungistatic against phytopathogen fungi or display signaling functions in soil and rhizosphere.[32]

4. Experimental section

4.1. General experimental procedures

Optical rotations were determined with a Perkin–Elmer 241 polarimeter. IR spectra were recorded on a Mattson Genesis spectrophotometer, series FTIR. ^{1}H and ^{13}C NMR measurements were obtained on Varian Unity 400 NMR spectrometers with $SiMe_4$ as the internal reference. Mass spectra were recorded on a GC–MS Thermoquest spectrometer (model: Voyager), and a VG Autospec-Q spectrometer. High-performance liquid chromatography (HPLC) was performed with a Hitachi/Merck L-6270 apparatus equipped with a UV–vis detector (L4250) and a differential refractometer detector (RI-71). Thin layer chromatography (TLC) was performed on Merck Kiesegel $60F_{254}$, 0.2 mm thick. Silica gel (Merck) was used for column chromatography. Purification by means of HPLC was accomplished with a silica gel column (Hibar 60, 7 m, 1 cm wide, 25 cm long). Racemic mixtures were separated by means of HPLC analysis on chiral column (Chiracel OD, Daicel, Japan). Chemicals were products of Fluka or Aldrich. All solvents used were freshly distilled.

4.2. Microorganism cultures

The cultures of *T. harzianum* CECT 2413 and *T. viride* UCA 06 employed in this study were obtained from the 'Colección Española

de Cultivos Tipo' (CECT), Facultad de Biología, Universidad de Valencia, Spain and from the mycological collection of the Microbiological Department, Universidad de Cádiz, respectively, where cultures of these strains are on deposit.

4.3. General culture conditions

Fungi were grown at 25 °C on a PDB medium (150 mL per Roux bottle) in 20 Roux bottles. Cultivation was carried out for 13 days under static conditions and artificial light (day light type). The mycelium was subsequently filtered and then washed with brine and ethyl acetate. The broth was extracted three times with ethyl acetate and the extract dried over anhydrous sodium sulfate. The solvent was then evaporated and the residue chromatographed first on a silica gel column and then compounds from different fractions were purified by HPLC.

4.4. Culture condition with elicitor

T. viride was grown at 25 °C on a PDB medium (150 mL per Roux bottle). After two days' growth, nicotinic acid dissolved in ethanol (150 ppm per flask) was then distributed over 61 Roux bottles as a secondary metabolite elicitor. Fermentation continued for five days, after which the mycelium was filtered and then washed with brine and ethyl acetate. The broth was extracted three times with ethyl acetate and the extract was dried over anhydrous sodium sulfate. The solvent was then evaporated and the residue treated as indicated in the general procedure.

4.5. Microorganism and antifungal assays

The *B. cinerea* 2100 employed in this work was obtained from the CECT, where a culture of this strain is on deposit. Bioassays were performed by measuring the inhibition of radial growth on an agar medium in a Petri dish. The test compound was dissolved in acetone yielding final compound concentrations of 100 mg L^{-1}. Solutions of the test compound were added to a glucose–malt–peptone–agar medium (61 g of glucose–malt–peptone–agar per liter, pH 6.5–7.0). The final acetone concentration was identical in both control and treated cultures. The medium was poured into sterile plastic Petri dishes measuring 9 cm in diameter and a mycelia disc of *B. cinerea* cut from an actively growing culture measuring 5 mm in diameter was placed in the center of the agar plate. Growth inhibition was calculated as the percentage of inhibition of radial growth relative to the control (*I*%) and was measured for seven days. The assays were carried out in triplicate and the results are shown as mean values of three replicates of colony diameters; (±SD). The commercial fungicide dichlofluanid was used throughout as a standard for comparison.

Compound	1	2	3	4	5	Control
%I/4d.	100±0	15±1	10±2	15±2	25±1	100±0
%I/7d.	64±2	6±1	—	7±1	15±2	100±0

4.6. Products isolated from *Thctf1* null mutant

The broth obtained from the fermentation of the null mutant was filtered with Nylon 200 μm filter and treated as indicated in the general culture procedure to yield a dense oil, which was chromatographed and the fractions obtained were collected and purified by high-pressure liquid chromatography (HPLC). Study of the fraction obtained by spectroscopic methods, specifically ^{1}H NMR and ^{13}C NMR, revealed the presence of fatty acids, triglycerides, and phthalate derivatives.

4.7. Products isolated from *T. viride* UCA06

Following the general culture procedure, after chromatography the extract yielded compounds (**1**) (500 mg), trichoviridin (**8**)[22] (97 mg), and tyrosol (**9**)[23] (13.7 mg).

From fermentation with nicotinic acid as the elicitor chromatography of the extract obtained from the broth yielded 6-pentyl-2*H*-pyran-2-one (**1**) (1.3 g), 6-(5- hydroxypentyl)-α-pyrone (**2**) (7.6 mg), 6-(4-hydroxypentyl)-2*H*-pyran-2-one (**3**) (1.6 mg),[16,18] viridepyronone (**4**)[21] (16 mg), and 6-((1′*S*,2′*R*)-2′-propyloxiran-1-yl)-2*H*-pyran-2-one (**7a**) (6 mg).

4.8. Products isolated from *T. harzianum*

Chromatography of the fermented extract gave 6-pentyl-2*H*-pyran-2-one (**1**) (223 mg), 6-(4-hydroxypentyl)-2*H*-pyran-2-one (**3**) (2.2 mg), and 6-[(1′*R*,2′*S*)-dihydroxypentyl]-2*H*-pyran-2-one (**5**) (4.3 mg).

*4.8.1. 6-[(1′R,2′S)-Dihydroxypentyl]-2H-pyran-2-one (**5**)*

$[\alpha]_D^{25}$ +122.5 (*c* 0.11, MeOH). IR (film): ν_{max}=3388, 1714, 1632, 1556, 1088, 812 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.34 (dd, *J*=6.4, 9.2 Hz, 1H, 4-H), 6.36 (d, *J*=6.4 Hz, 1H, 5-H), 6.18 (d, *J*=9.2 Hz, 1H, 3-H), 4.24 (br s, 1H, 1′-H), 3.98 (m, 1H, 2′-H), 1.48 (m, 4H, 3′-H, 4′-H), 0.93 (t, *J*=7.0 Hz, 3H, 5′-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ=165.1 (s, C-2), 162.2 (s, C-6), 143.7 (d, C-4), 114.2 (d, C-3), 102.9 (d, C-5), 73.2 (d, C-1′), 71.8 (d, C-2′), 35.4 (t, C-3′), 18.9 (t, C-4′), 13.9 (q, C-5′) ppm. MS: *m*/*z*=180 (2) $[M-H_2O]^+$, 130 (11), 97 (8), 55 (5). HRMS calcd. for $C_{10}H_{14}O_4$: 198.0888; found: 199.1018 $[M+1]^+$.

*4.8.2. 6-[(1′R,2′S)-Diacetoxypentyl]-2H-pyran-2-one (**6**)*

Compound (**5**) (3 mg) and acetic anhydride (1 mL) were dissolved in dry pyridine (330μL) and the mixture was stirred 26 h at room temperature. The excess of acetic anhydride was then eliminated with cyclohexane and acetone and evaporated in a rotary. The residue was purified by column chromatography using hexane-EtOAc (10%) as an eluent to give **6** in a 90% yield as an oil; ^{1}H NMR (400 MHz, $CDCl_3$): 7.29 (1H, dd, *J*=6.4, 9.5 Hz, H-4), 6.24 (1H, d, *J*=9.5 Hz, H-5), 6.20 (1H, d, *J*=6.4 Hz, H-3), 5.56 (1H, d, *J*=4.6 Hz, H-1′), 5.28 (1H, ddd, *J*=4.6, 8.5, 4.6 Hz, H-2′), 2.14 (3H, s, C*H*$_3$COO-), 2.03 (3H, s, C*H*$_3$COO–), 1.30 (4H, m, H-2′ y H-3′), δ=0.90 (3H, t, *J* =7.4 Hz, H-5′); RMN ^{13}C (100 MHz, $CDCl_3$) δ=170.1 (s, CH_3*C*OO–), 169.4 (s, CH_3*C*OO–), 160.9 (s, C-2), 159.4 (s, C-6), 142.7 (d, C-4), 115.6 (d, C-3), 103.8 (d, C-5), 72.2 (d, C*-1′), 72.1 (d, C*-2′), 31.7 (t, C-3′), 20.8 (c, *C*H$_3$COO-), 20.7 (c, *C*H$_3$COO-), 18.5 (t, C-4′), 13.7 (c, C-5′), *=interchangeable.

*4.8.3. 6-[(1′S,2′R)-2′-Propyloxiran-1-yl]-2H-pyran-2-one (**7a**)*

$[\alpha]_D^{25}$ −90.0 (*c* 0.45, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.27 (dd, *J*=6.7, 9.5 Hz, 1H, 4-H), 6.22 (d, *J*=9.5 Hz, 1H, 3-H), 6.18 (d, *J*=6.7 Hz, 1H, 5-H), 3.36 (d, *J*=2.0 Hz, 1H, 1′-H), 3.17 (ddd, *J*=2.0, 5.1, 6.1 Hz, 1H, 2′-H), 1.62 (m, *J*=5.1 Hz, 2H, 3′-H), 1.51 (m, *J*=7.4 Hz, 2H, 4′-H), 0.97 (t, *J*=7.4 Hz, 3H, 5′-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ=161.3 (s, C-6), 161.2 (s, C-2), 143.1 (d, C-4), 115.2 (d, C-3), 102.3 (d, C-5), 61.1 (d, C-2′), 54.3 (d, C-1′), 33.7 (t, C-3′), 19.0 (t, C-4′), 13.8 (q, C-5′) ppm. MS: *m*/*z*=180 (2) $[M]^+$, 126 (100), 97 (7), 95 (10). HRMS calcd. for $C_{10}H_{12}O_3$: 180.0783; found: 181.0866 $[M+1]^+$.

4.9. Hemisynthesis of enantiomeric pure compounds 6-[(1′*S*,2′*R*)-2′-propyloxiran-1-yl]-2*H*-pyran-2-one (7a) and 6-[(1′*R*,2′*S*)-2′-propyloxiran-1-yl]-2*H*-pyran-2-one (7b)

*4.9.1. (±) 6-(2′-Propyloxiran-1-yl)-2H-pyran-2-one (**7**)*

m-CPBA (77%, 2.94 g, 13.15 mmol) was added to a solution of the olefin **11** (1.8 g, 10.96 mmol) in CH_2Cl_2 (150 mL) at 0 °C and the mixture warmed up to room temperature (24 °C) within 14 h. A

solution of Na_2SO_3 (40 mL) was added and the aqueous phase was extracted with CH_2Cl_2 (4×20 mL), the combined organic phases were washed with $NaHCO_3$ (40 mL) and brine (40 mL), dried with Na_2SO_4, and concentrated in vacuo. The residue was purified by flash chromatography (hexane/ethyl acetate, 85:15) to obtain 1.73 g (81%) of the desired racemic epoxide **7** as a colorless oil whose spectral data were identical to those isolated from *T. viride*. The racemic mixture of epoxide **7** was separated by means of HPLC analyses on a chiral column (Chiralcel OD, Daicel, Japan): 254 nm, 0.5 mL/min, hexane/isopropanol (9.5:0.5) yielding enantiomerically pure epoxides **7a** $[\alpha]_D^{25}$ −91.6 (*c* 0.55, $CHCl_3$) and **7b** $[\alpha]_D^{25}$ +93.8 (*c* 0.56, $CHCl_3$).

Epoxide **7a** (3 mg) in dimethoxyethane (DME) (1 mL) was treated with perchloric acid (0.25 mL, 6% in water) at 0 °C. The reaction mixture was stirred for 6 h at room temperature (24 °C) and then poured into water (2 mL) and extracted with 2 mL of ether. The ether extracts were washed with saturated solution of bicarbonate sodium and water, dried over $MgSO_4$, and concentrated. The resulting oil was chromatographed yielding 6-[(1′*R*,2′*R*)-dihydroxypentyl]-2*H*-pyran-2-one (**15**) (2 mg) $[\alpha]_D^{25}$ +81.75 (*c* 0.15, MeOH).

4.10. Hemisynthesis of enantiomeric pure compounds 12–15

*4.10.1. 6-(1′-Bromopentyl)-2H-pyran-2-one (**10**)*

Compound **1** (1 g, 6.02 mmol), NBS (2.14 g, 7.2 mmol), and benzoyl peroxide (145 mg, 0.6 mmol) were dissolved in CCl_4 (18 mL) and then refluxed at 90 °C for 12 h. The reaction mixture was cooled to room temperature, diluted with water, extracted with Et_2O, and the combined organic phases were washed with brine, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. Purification of crude product by flash column chromatography (silica gel, 15% EtOAc in hexane, gradient elution) provided the desired product **10** (1.43 g, 97%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.22 (dd, *J*=6.6, 9.3 Hz, 1H, 4-H), 6.17 (d, *J*=9.3 Hz, 1H, 3-H), 6.15 (d, *J*=6.6 Hz, 1H, 5-H), 4.50 (t, *J*=7.7 Hz, 1H, 1′-H), 2.07 (m, 2H, 2′-H), 1.32 (m, 1H, 3′a-H), 1.26 (m, 3H, 3′b-H, 4′-H), 0.83 (t, *J*=6.9 Hz, 3H, 5′-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ=162.3 (s, C-6), 161.1 (s, C-2), 142.9 (d, C-4), 115.8 (d, C-3), 103.1 (d, C-5), 48.0 (d, C-1′), 35.4 (t, C-2′), 29.7 (t, C-3′), 21.8 (t, C-4′), 13.7 (q, C-5′) ppm. MS: *m*/*z*=165 (100), 244 (30), 246 (20). HRMS calcd. for $C_{10}H_{13}BrO_2$: 244.1125; found: 244.0116.

*4.10.2. 6-(Pent-1′-enyl)-2H-pyran-2-one (**11**)[25]*

LiBr (2.1 g, 24.47 mmol) and Li_2CO_3 (1.8 g, 24.47 mmol) were added to a solution of **10** (1 g, 4.08 mmol) in DMF (35 mL) and the mixture was heated in an oil bath at 110 °C under argon atmosphere for 1 h. After cooling, H_2O was added and the reaction mixture was extracted three times with Et_2O. The organic layer was dried ($MgSO_4$) and evaporated under vacuum. The residue was purified by flash column chromatography (silica gel, 15% EtOAc in hexane, gradient elution) to afford the desired product **11** (0.5 g, 75%), whose spectroscopic data coincided with those reported previously.

*4.10.3. threo-Diols 6-(1′,2′-dihydroxypentyl)-2H-pyran-2-one racemic mixture (**12**+**13**)*

The dihydroxylation of the double bond was conducted under standard catalytic conditions. Trimethylamine *N*-oxide dihydrate (1.35 g, 12 mmol) and osmium tetraoxide (77 mg, 0.3 mmol) were added to a solution of olefin **11** (1 g, 6 mmol) in *t*-BuOH/H_2O (2/1, 25 mL). After 2 h of refluxing, the mixture was cooled to room 25 °C and partitioned between brine (30 mL) and ethyl acetate (40 mL). The organic layer was separated and the aqueous one extracted with ethyl acetate (4×20 mL), and the combined organic solutions were dried and concentrated. Purification of the crude product by column chromatography (hexane/ethyl acetate, 1:1) afforded the title racemic *threo*-diols (**12**+**13**) as an oil (0.96 g, 80%) whose spectral data were identical to those of the natural compound **5**.

*4.10.4. erythro-Diols 6-(1′,2′-dihydroxypentyl)-2H-pyran-2-one racemic mixture (**14**+**15**)*

Perchloric acid (0.5 mL, 6% in water) at 0 °C was added to a solution of the epoxide **7** (295 mg, 1.64 mmol) in dimethoxyethane (DME) (5 mL). The reaction mixture was stirred for 8 h at room temperature (24 °C) and then poured into water (20 mL) and extracted with 25 mL (3×) of ether. The combined ether extracts were washed with a saturated solution of bicarbonate sodium and water, dried over $MgSO_4$, and concentrated. The resulting oil was chromatographed on silica gel with hexane and ethyl acetate to afford the racemic mixture of **14**+**15** (227 mg, 70%) and the rearranged compound **16** (59 mg, 20%).

*4.10.5. 6-(1′,2′-Dihydroxypentyl)-2H-pyran-2-one racemic mixture (**14**+**15**)*

IR (film): ν_{max}=3383, 1710, 1632, 1560, 1091, 812 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.32 (dd, *J*=6.7, 9.5 Hz, 1H, 4-H), 6.33 (d, *J*=6.7 Hz, 1H, 5-H), 6.11 (d, *J*=9.5 Hz, 1H, 3-H), 4.38 (d, *J*=4.1 Hz, 1H, 1′-H), 3.87 (m, 1H, 2′-H), 1.35 (m, 4H, 3′-H, 4′-H), 0.80 (t, *J*=7.2 Hz, 3H, 5′-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ=164.7 (s, C-2), 162.7 (s, C-6), 144.2 (d, C-4), 113.8 (d, C-3), 103.3 (d, C-5), 73.6 (d, C-1′), 72.6 (d, C-2′), 33.1 (t, C-3′), 18.7 (t, C-4′), 13.8 (q, C-5′) ppm. MS: *m*/*z*=162 (2) $[M-2H_2O]^+$, 130 (11), 126 (100), 97 (16), 55 (12).

*4.10.6. 6-(2-Oxopentyl)-2H-pyran-2-one (**16**)*

IR (film): ν_{max}=1724, 1636, 1377, 1098, 807 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.26 (dd, *J*=6.7, 9.5 Hz, 1H, 4-H), 6.16 (d, *J*=9.5 Hz, 1H, 3-H), 6.08 (d, *J*=6.7 Hz, 1H, 5-H), 3.52 (s, 2H, 1′-H), 2.46 (t, *J*=7.4 Hz, 2H, 3′-H), 1.55 (q, *J*=7.4 Hz, 2H, 4′-H), 0.86 (t, *J*=7.4 Hz, 3H, 5′-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ=203.8 (s, C-2′), 162.3 (s, C-6), 158.1 (s, C-2), 143.7 (d, C-4), 113.9 (d, C-3), 104.5 (d, C-5), 48.2 (t, C-1′), 44.1 (t, C-3′), 16.7 (t, C-4′), 14.4 (q, C-5′) ppm. MS: *m*/*z*=180 (12), 137 (5), 110 (100), 95 (14), 71 (45). HRMS calcd. for $C_{10}H_{12}O_3$: 180.0783; found: 180.1775 $[M]^+$.

4.11. Mosher's diesters: general procedure

DMAP (6.2 mg, 0.05 mmol), DCC (147.9 mg, 0.55 mmol), and (*R*)-MPA or (*S*)-MPA (92.3 g, 0.55 mmol) were added to a stirred solution of *threo*- and *erythro*-diol racemic mixture (50 mg, 0.25 mmol) in dichloromethane (1 mL) at room temperature. The resulting mixture was stirred for 48 h. Precipitates were removed by filtration over Celite and washed with pentane (5×6 mL). The filtrate was subsequently washed with a 1.0 M aqueous HCl solution (2×3 mL), a saturated aqueous $NaHCO_3$ solution (2×3 mL), and a saturated aqueous NaCl solution (3 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, hexane/ethyl acetate 70:30) followed by HPLC purification (hexane/ethyl acetate 70:30) to afford the desired compounds **12a**–**15a** and **12b**–**15b** with an average yield of 30%.

*4.11.1. Bis-(R)-MPA ester of 6-[(1′R,2′S)-dihydroxypentyl]-2H-pyran-2-one (**12a**)*

$[\alpha]_D^{25}$ +14.7 (*c* 0.17, MeOH). IR (film): ν_{max}=1747, 1643, 1560, 1167, 1112, 805 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.30–7.42 (m, 10H, ArH), 6.76 (dd, *J*=6.5, 9.5 Hz, 1H, 4-H), 6.02 (d, *J*=9.5 Hz, 1H, 3-H), 5.49 (d, *J*=4.6 Hz, 1H, 1′-H), 5.29 (dt, *J*=4.6, 9.2 Hz, 1H, 2′-H), 5.17 (d, *J*=6.5 Hz, 1H, 5-H), 4.75 (s, 1H, MPA-H), 4.66 (s, 1H, MPA-H), 3.38 (s, 3H, OMe), 3.35 (s, 3H, OMe), 1.28–1.49 (m, 2H, 3′-H), 0.94–1.04 (m, 2H, 4′-H), 0.69 (t, *J*=7.3 Hz, 3H, 5′-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ=169.7 (s, CO-MPA), 169.0 (s, CO-MPA), 160.4 (s, C-2), 158.2

(s, C-6), 142.1 (d, C-4), 136.1, 135.4, 129.1, 128.7, 128.7, 128.5, 127.4, 127.1 (8d, Benzyl-MPA), 115.4 (d, C-3), 102.7 (d, C-5), 82.5, 81.9 (2d, CH-MPA), 72.3 (d, C-1′), 72.1 (d, C-2′), 57.3 (s, OMe), 57.2 (s, OMe), 32.1 (t, C-3′), 17.8 (t, C-4′), 13.4 (q, C-5′) ppm. MS: m/z=494 (5), 404 (33), 292 (87), 268 (41), 218 (35), 230 (52), 130 (100), 95 (14), 71 (45). HRMS calcd. for $C_{28}H_{30}O_8$: 494.1940; found: 494.1947 $[M]^+$.

*4.11.2. Bis-(R)-MPA ester of 6-[(1′S,2′R)-dihydroxypentyl]-2H-pyran-2-one (**13a**)*

$[\alpha]_D^{25}$ −117.6 (*c* 0.28, MeOH). IR (film): ν_{max}=1751, 1639, 1560, 1165, 1112, 804 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ=7.34–7.46 (m, 10H, ArH), 6.70 (dd, *J*=6.7, 9.2 Hz, 1H, 4-H), 5.97 (d, *J*=9.2 Hz, 1H, 3-H), 5.39 (d, *J*=2.6 Hz, 1H, 1′-H), 5.24 (ddd, *J*=2.6, 4.6, 8.7 Hz, 1H, 2′-H), 5.12 (d, *J*=6.7 Hz, 1H, 5-H), 4.78 (s, 1H, MPA-H), 4.62 (s, 1H, MPA-H), 3.38 (s, 3H, OMe), 3.29 (s, 3H, OMe), 1.08–1.26 (m, 2H, 3′-H), 1.10 (m, 2H, 4′-H), 0.69 (t, *J*=7.0 Hz, 3H, 5′-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ=169.3 (s, CO-MPA), 169.1 (s, CO-MPA), 160.5 (s, C-2), 158.0 (s, C-6), 142.2 (d, C-4), 136.3, 135.5, 129.1, 128.7, 128.6, 128.4, 127.3, 127.1 (8d, Benzyl-MPA), 114.9 (d, C-3), 102.3 (d, C-5), 82.1, 81.9 (2d, CH-MPA), 72.1 (d, C-1′), 71.6 (d, C-2′), 57.3 (s, OMe), 57.2 (s, OMe), 32.0 (t, C-3′), 18.2 (t, C-4′), 13.4 (q, C-5′) ppm. HRMS calcd. for $C_{28}H_{30}O_8$: 494.1940; found: 494.1988 $[M]^+$.

*4.11.3. Bis-(S)-MPA ester of 6-[(1′R,2′S)-dihydroxypentyl]-2H-pyran-2-one (**12b**)*

$[\alpha]_D^{25}$ +120.5 (*c* 0.19, MeOH). IR (film): ν_{max}=1748, 1643, 1560, 1165, 1106, 803 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ=7.34–7.46 (m, 10H, ArH), 6.70 (dd, *J*=6.7, 9.2 Hz, 1H, 4-H), 5.97 (d, *J*=9.2 Hz, 1H, 3-H), 5.40 (d, *J*=2.6 Hz, 1H, 1′-H), 5.24 (ddd, *J*=2.6, 4.6, 8.7 Hz, 1H, 2′-H), 5.12 (d, *J*=6.7 Hz, 1H, 5-H), 4.78 (s, 1H, MPA-H), 4.62 (s, 1H, MPA-H), 3.38 (s, 3H, OMe), 3.29 (s, 3H, OMe), 1.08–1.26 (m, 2H, 3′-H), 1.10 (m, 2H, 4′-H), 0.69 (t, *J*=7.0 Hz, 3H, 5′-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ=169.3 (s, CO-MPA), 169.1 (s, CO-MPA), 160.5 (s, C-2), 158.0 (s, C-6), 142.2 (d, C-4), 136.3, 135.5, 129.1, 128.7, 128.6, 128.4, 127.3, 127.1 (8d, Benzyl-MPA), 114.9 (d, C-3), 102.3 (d, C-5), 82.1, 81.9 (2d, CH-MPA), 72.1 (d, C-1′), 71.6 (d, C-2′), 57.3 (s, OMe), 57.2 (s, OMe), 32.0 (t, C-3′), 18.2 (t, C-4′), 13.4 (q, C-5′) ppm. HRMS calcd. for $C_{28}H_{30}O_8$: 494.1940; found: 494.1956 $[M]^+$.

*4.11.4. Bis-(S)-MPA ester of 6-[(1′S,2′R)-dihydroxypentyl]-2H-pyran-2-one (**13b**)*

$[\alpha]_D^{25}$ −12.4 (*c* 0.24, MeOH). IR (film): ν_{max}=1747, 1642, 1560, 1167, 1113, 805 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ=7.30–7.42 (m, 10H, ArH), 6.76 (dd, *J*=6.5, 9.5 Hz, 1H, 4-H), 6.02 (d, *J*=9.5 Hz, 1H, 3-H), 5.49 (d, *J*=4.6 Hz, 1H, 1′-H), 5.30 (dt, *J*=4.6, 9.0 Hz, 1H, 2′-H), 5.17 (d, *J*=6.5 Hz, 1H, 5-H), 4.76 (s, 1H, MPA-H), 4.66 (s, 1H, MPA-H), 3.39 (s, 3H, OMe), 3.35 (s, 3H, OMe), 1.28–1.49 (m, 2H, 3′-H), 0.94–1.04 (m, 2H, 4′-H), 0.69 (t, *J*=7.3 Hz, 3H, 5′-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ=169.7 (s, CO-MPA), 169.0 (s, CO-MPA), 160.4 (s, C-2), 158.2 (s, C-6), 142.1 (d, C-4), 136.1, 135.4, 129.1, 128.7, 128.7, 128.5, 127.4, 127.1 (8d, Benzyl-MPA), 115.4 (d, C-5), 102.7 (d, C-3), 82.5, 81.9 (2d, CH-MPA), 72.3 (d, C-1′), 72.1 (d, C-2′), 57.3 (s, OMe), 57.2 (s, OMe), 32.1 (t, C-3′), 17.8 (t, C-4′), 13.4 (q, C-5′) ppm. HRMS calcd. for $C_{28}H_{30}O_8$: 494.1940; found: 494.1946 $[M]^+$.

*4.11.5. Bis-(R)-MPA ester of 6-[(1′S,2′S)-dihydroxypentyl]-2H-pyran-2-one (**14a**)*

$[\alpha]_D^{25}$ −97.9 (*c* 0.24, MeOH). IR (film): ν_{max}=1750, 1641, 1560, 1165, 1107, 805 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ=7.26–7.44 (m, 10H, ArH), 7.10 (dd, *J*=6.5, 9.3 Hz, 1H, 4-H), 6.16 (d, *J*=9.3 Hz, 1H, 3-H), 5.97 (d, *J*=6.5 Hz, 1H, 5-H), 5.52 (d, *J*=6.4 Hz, 1H, 1′-H), 5.15 (ddd, *J*=3.6, 6.4, 9.5 Hz, 1H, 2′-H), 4.79 (s, 1H, MPA-H), 4.51 (s, 1H, MPA-H), 3.37 (s, 3H, OMe), 3.33 (s, 3H, OMe), 1.01–1.18 (m, 2H, 3′-H), 0.73 (m, 2H, 4′-H), 0.48 (t, *J*=7.3 Hz, 3H, 5′-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ=169.4 (s, CO-MPA), 169.0 (s, CO-MPA), 160.5 (s, C-2), 158.0 (s, C-6), 142.4 (d, C-4), 135.8, 135.4, 128.9, 128.6, 128.5, 128.3, 127.1, 126.8 (8d, Benzyl-MPA), 115.7 (d, C-3), 104.3 (d, C-5), 82.1, 81.9 (2d, CH-MPA), 72.2 (d, C-1′), 71.7 (d, C-2′), 57.3 (s, OMe), 57.2 (s, OMe), 30.8 (t, C-3′), 17.2 (t, C-4′), 13.1 (q, C-5′) ppm. HRMS calcd. for $C_{28}H_{30}O_8$: 494.1940; found: 494.1950 $[M]^+$.

*4.11.6. Bis-(R)-MPA ester of 6-[(1′R,2′R)-dihydroxypentyl]-2H-pyran-2-one (**15a**)*

$[\alpha]_D^{25}$ +14.8 (*c* 0.26, MeOH). IR (film): ν_{max}=1744, 1638, 1560, 1168, 1104, 805 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ=7.25–7.36 (m, 10H, ArH), 6.76 (dd, *J*=6.4, 9.2 Hz, 1H, 4-H), 6.00 (d, *J*=9.2 Hz, 1H, 3-H), 5.41 (d, *J*=4.6 Hz, 1H, 1′-H), 5.36 (dt, *J*=4.6, 6.4 Hz, 1H, 2′-H), 5.04 (d, *J*=6.4 Hz, 1H, 5-H), 4.67 (s, 1H, MPA-H), 4.62 (s, 1H, MPA-H), 3.37 (s, 3H, OMe), 3.33 (s, 3H, OMe), 1.56 (m, *J*=7.4, 6.4 Hz, 2H, 3′-H), 1.22–1.33 (m, 2H, H-4′), 0.86 (t, *J*=7.3 Hz, 3H, 5′-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ=169.6 (s, CO-MPA), 168.7 (s, CO-MPA), 160.4 (s, C-2), 157.6 (s, C-6), 142.3 (d, C-4), 136.0, 135.3, 128.8, 128.7, 128.6, 127.3, 127.1 (8d, Benzyl-MPA), 115.2 (d, C-3), 103.7 (d, C-5), 82.1, 81.8 (2d, CH-MPA), 72.4 (d, C-1′), 72.1 (d, C-2′), 57.4 (s, OMe), 57.2 (s, OMe), 31.9 (t, C-3′), 18.3 (t, C-4′), 13.5 (q, C-5′) ppm. HRMS calcd. for $C_{28}H_{30}O_8$: 494.1940; found: 494.1923 $[M]^+$.

*4.11.7. Bis-(S)-MPA ester of 6-[(1′S,2′S)-dihydroxypentyl]-2H-pyran-2-one (**14b**)*

$[\alpha]_D^{25}$ −13.4 (*c* 0.26, MeOH). IR (film): ν_{max}=1744, 1638, 1560, 1169, 1105, 805 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ=7.26–7.37 (m, 10H, ArH), 6.76 (dd, *J*=6.7, 9.2 Hz, 1H, 4-H), 6.01 (d, *J*=9.2 Hz, 1H, 3-H), 5.41 (d, *J*=4.6 Hz, 1H, 1′-H), 5.37 (dt, *J*=4.6, 6.4 Hz, 1H, 2′-H), 5.05 (d, *J*=6.7 Hz, 1H, 5-H), 4.68 (s, 1H, MPA-H), 4.64 (s, 1H, MPA-H), 3.39 (s, 3H, OMe), 3.35 (s, 3H, OMe), 1.58 (m, *J*=7.4, 6.4 Hz, 2H, 3′-H), 1.22–1.33 (m, 2H, 4′-H), 0.87 (t, *J*=7.3 Hz, 3H, 5′-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ=169.4 (s, CO-MPA), 168.6 (s, CO-MPA), 160.2 (s, C-2), 157.4 (s, C-6), 142.2 (d, C-4), 135.9, 135.1, 128.7, 128.6, 128.4, 127.2, 127.0 (8d, Benzyl-MPA), 115.0 (d, C-3), 103.6 (d, C-5), 82.0, 81.7 (2d, CH-MPA), 72.2 (d, C-1′), 72.0 (d, C-2′), 57.2 (s, OMe), 57.0 (s, OMe), 31.7 (t, C-3′), 18.1 (t, C-4′), 13.3 (q, C-5′) ppm. HRMS calcd. for $C_{28}H_{30}O_8$: 494.1940; found: 494.1953 $[M]^+$.

*4.11.8. Bis-(S)-MPA ester of 6-[(1′R,2′R)-dihydroxypentyl]-2H-pyran-2-one (**15b**)*

$[\alpha]_D^{25}$ +96.1 (*c* 0.22, MeOH). IR (film): ν_{max}=1748, 1644, 1562, 1166, 1113, 806 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ=7.28–7.44 (m, 10H, ArH), 7.10 (dd, *J*=6.5, 9.3 Hz, 1H, H-4), 6.17 (d, *J*=9.3 Hz, 1H, H-3), 5.98 (d, *J*=6.5 Hz, 1H, H-5), 5.52 (d, *J*=6.4 Hz, 1H, H-1′), 5.15 (ddd, *J*=3.6, 6.4, 9.5 Hz, 1H, H-2′), 4.80 (s, 1H, H-MPA), 4.53 (s, 1H, H-MPA), 3.38 (s, 3H, OMe), 3.34 (s, 3H, OMe), 1.02–1.17 (m, 2H, H-3′), 0.74 (m, 2H, H-4′), 0.49 (t, *J*=7.3 Hz, 3H, H-5′) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ=169.4 (s, CO-MPA), 169.0 (s, CO-MPA), 160.5 (s, C-2), 158.0 (s, C-6), 142.4 (d, C-4), 135.8, 135.4, 128.9, 128.6, 128.5, 128.3, 127.1, 126.8 (8d, Benzyl-MPA), 115.7 (d, C-3), 104.3 (d, C-5), 82.1, 81.9 (2d, CH-MPA), 72.2 (d, C-1′), 71.7 (d, C-2′), 57.3 (s, OMe), 57.2 (s, OMe), 30.8 (t, C-3′), 17.2 (t, C-4′), 13.1 (q, C-5′) ppm. HRMS calcd. for $C_{28}H_{30}O_8$: 494.1940; found: 494.1950 $[M]^+$.

4.12. Hydrolysis of Mosher's esters: general procedure

The corresponding Mosher's ester (10 mg, 0.02 mmol) was dissolved in methanol (5 mL). Then three drops of an aqueous solution of K_2CO_3 (6.1 mg, 0.044 mmol) were added and the mixture was stirred at room temperature for 3 min. The reaction mixture was directly chromatographed on flash chromatography column (silica gel, hexane/ethyl acetate 2:3) to yield the desired product 6-[(1′*R*,2′*S*)-dihydroxypentyl]-2*H*-pyran-2-one (**12**) (76%), $[\alpha]_D^{25}$ +120.6 (*c* 0.15, MeOH); 6-[(1′*S*,2′*R*)-dihydroxypentyl]-2*H*-pyran-2-one (**13**) (75%), $[\alpha]_D^{25}$ −123.1 (*c* 0.1, MeOH); 6-[(1′*S*,2′*S*)-dihydroxypentyl]-2*H*-pyran-2-one (**14**) (80%), $[\alpha]_D^{25}$ −79.6 (*c* 0.5,

MeOH); or 6-[(1′*R*,2′*R*)-dihydroxypentyl]-2*H*-pyran-2-one (**15**) (78%), $[\alpha]_D^{25}$ +81.3 (*c* 0.13, MeOH).

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References and notes

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